THE EFFECTS OF CHLORDANE ON PREGNANT MICE AND THEIR OFFSPRING

A Thesis

Presented to

The Faculty of the School of Sciences and Mathematics

Morehead State University

In Partial Fulfillment

Of the Requirements for the Degree

Master of Science in Biology

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by

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May 1973

Accepted by the faculty of the School of Sciences and Mathematics, in partial fulfillment of the requirements for the Master of Science degree.

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ABSTRACT OF THESIS

THE EFFECTS OF CHLORDANE ON PREGNANT MICE AND THEIR OFFSPRING

Pregnant mice were randomly isolated into one of nine different groups and injected with specific concentrations of chlordane at different periods of gestation: either early, middle, or late gestation.

There were three major objectives. The primary overt objective was to define possible embryonic or postnatal offspring abnormalities resulting from intraperitoneal administrations of varying concentrations of chlordane to pregnant Swiss Webster mice. A correlated objective was to describe sibling and maternal behavioral and survival parameters. A third objective was to determine sublethal and lethal doses of chlordane under varying conditions.

Recorded observations included the survival rates of pregnant mice, a comparison of the litter sizes, the survival rates of mouse pups until weaning, and the frequency of maternal cannibalism.

It was concluded that maternal survival was adversely affected when the chlordane treatments were administered during early gestation; that sibling survival rates were adversely affected when the chlordane treatments

were administered during the early and middle periods of gestation; that litter size and frequency of maternal cannibalism were not affected by the chlordane treatments; and that no teratogenic defects were observed among the offspring whose maternal parents received chlordane treatments.

ACKNOWLEDGMENTS

I am deeply indebted to Dr. Jerry F. Howell, Jr., the chairman of my graduate committee, who freely gave of his time during the entire experiment. Dr. Howell's advice and assistance throughout this research, including the preparation of this manuscript, were immeasurable. I feel fortunate to have had an individual of such high caliber as chairman of my committee.

I would also like to thank the other members of my committee, Dr. Madison E. Pryor and Dr. James R. Spears, for their interest and constructive criticism concerning this research.

I am especially indebted to Mr. Henry D. Muse, Assistant Professor of Mathematics, for his assistance in designing the statistical analyses that were utilized in evaluating the results of this study.

I would also like to thank the Psychology Department for providing extra materials to maintain the animals used in this research.

My very special thanks go to my wife, Gail, who typed this entire manuscript. Her deep concern and good nature were instrumental in the successful outcome of this project.

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CHAPTER I

INTRODUCTION

Importance of the Subject

The widespread use of insecticides during the past thirty years has prompted prolific scientific investigations concerned with the physiological and ecological effects of these biocides. The detrimental effects of insecticides have been summarized by such authors as Rudd (1964), Carson (1962), and the U.S. Department of Health, Education, and Welfare (1969).

The commercial proliferation of allied formulations for various uses has fostered basic research. The persistent nature of chlorinated hydrocarbons and their allies has fostered studies on the long term effects of these pesticides. The Environmental Protection Agency recently banned most uses of dichlorodiphenyl trichloro-ethane (D.D.T.) (Gillette, 1972).

The production and use of chlordane, a chlorinated hydrocarbon closely related to D.D.T., is expected to increase because of the recent D.D.T. ban. For this reason and because present knowledge concerning the effects

of insecticides on pregnant mammals and their offspring is limited, the selection of this subject is especially timely.

Objectives of the Research

There were three major objectives of this research. The primary overt objective was to define possible embryonic or postnatal offspring abnormalities resulting from the intraperitoneal administration of varying concentrations of chlordane to pregnant Swiss Webster mice. A correlated objective was to describe sibling and maternal behavioral and survival parameters. A third objective was to determine sublethal and lethal doses of chlordane under varying conditions.

Scope of the Research

This research was not intended to be a comprehensive study on all possible effects of chlordane on mice; rather it was intended to delineate the insecticide's effects on maternal and sibling mice.

Recorded observations included the survival rates of pregnant mice, a comparison of the litter sizes, the survival rates of mouse pups until weaning and the frequency of maternal cannibalism. The results of these observations are herein recorded, substantiated by similar findings of previous researchers.

CHAPTER II

REVIEW OF THE LITERATURE

Chlordane

Chlordane, a chlorinated hydrocarbon with the empirical formula $C_{10}H_6Cl_8$, was synthesized by the Velsicol Corporation of Chicago, Illinois in 1945 (Kearns and Ingles, 1945). Chlordane, like other chlorinated hydrocarbon insecticides, such as DDT, aldrin, and dieldrin, is very persistent (Rudd, 1964). Lichtenstein and Polivka (1959) reported that 15 percent of a sprayed chlordane compound was detected in the soil 12 years after it was applied. Chlordane is absorbed through the skin, breathed in as a spray of dust, or absorbed in the digestive tract when swallowed (Carson, 1962). Chlordane is a cumulative poison and, like all other chlorinated hydrocarbons, its deposits accumulate in the body. A diet that contains as little as 2.5 parts per million (ppm) may lead to an accumulation of 75 ppm in the fat of experimental animals (Carson, 1962). Carson also quotes Dr. Arnold Lehman (1950), Chief Pharmacologist of the Food and Drug Administration: "chlordane is ... one of the most toxic insecticides--anyone handling it could be poisoned."

The major emphasis of the early toxicological research was concerned mainly with the acute toxicity of the new chemical. This chlorinated hydrocarbon insecticide was found to be more toxic than DDT; thus it remains most effective in the destruction of various species of insects (Kearns and Ingles, 1945). Approximately 50 percent of chlordane produced is estimated to be used in the pest control market for structural protection against termites (U.S. Department of Health, Education, and Welfare, 1969).

Ingles (1945) reported on the toxicity and mammalian effects of chlordane and DDT on laboratory rats. Various routes were used for insecticide administration, and he concluded that both insecticides had similar toxicities in rats. Ingles (1945) also noted that liver damage was less in rats subjected to chlordane than in those subjected to equal amounts of DDT, whereas pulmonary damage was more noticeable in the chlordane treated animals.

Stohlman, Thorp and Smith (1950) compared chlordane and DDT toxicity in rabbits. In this study, single and multiple insecticide doses were administered orally through a stomach tube, or a semisynthetic diet. It was concluded from the data that acute chlordane toxicity was slightly less than DDT in rats, and nearly equally toxic in rabbits, whereas chronic chlordane toxicity was considerably greater than DDT for both rats and rabbits.

During the early periods of its existence, chlordane had been used to control external parasites of domestic animals. Radeleff (1948) and Bushland, Wells, and Radeleff (1948) reported on the acute toxicity of chlordane sprays and dips and their effects on various forms of livestock. They concluded that chlordane should be used with extreme care. Rosenburg and Adler (1950) demonstrated that chicks fed chlordane died earlier and in a shorter time span than chicks fed DDT.

In the past twenty years, research has been centered on the effects of insecticides on mammalian organ systems. It was reported by Hart, Shultice and Fouts (1962) that, when sublethal doses of chlordane were administered by intraperitoneal injections to adult and weanling rats, hepatic microsomal activity for the metabolism of certain drugs was stimulated. There was no immediate hepatic microsomal activity following chlordane injections; however, increased activity was noticed about the eighth day following administration. They concluded that the delay period was a result of the physical properties of chlordane and the manner in which it was metabolized. Chlordane and its metabolites are insoluble in water. It was believed that they were stored in adipose tissue and gradually released in the body over a period of time. Davidow (1951) and Davidow and Radomski (1953) have shown that chlordane and its metabolites are stored in adipose

tissue following single or multiple administration. Stohlman et al. (1950) reported increasing organic chlorides in the urine of rabbits within 24 hours after administration of as little as 10 milligrams per kilogram (mg/kg). The organic chloride excretion peak was reached in two to three days after the administration of single doses of chlordane, but small amounts were in the urine several days thereafter.

Conney et al. (1965) postulated that various drugs induce changes in steroid metabolism, and reported that intraperitoneal administration of 10 mg/kg of chlordane to adult female rats every other day for 14 days resulted in a 385 percent increase in the metabolism of estradiol 17β to polar metabolites. Ambrose et al. (1953) reported that chlordane appeared to interfere with fertilization and lactation in rats that were fed a diet containing 0.032% chlordane from the time that they were weaned.

Related Compounds

The chemical structures and biological effects of many chlorinated hydrocarbons are similar. Reviewing the toxicological effects of other insecticides results in a better understanding of the possible effects of chlordane on pregnant mice.

Backstrom, Hansson, and Ullberg (1964) utilized C^{14} labeled DDT on pregnant mice and observed that large amounts of DDT were concentrated in the brain and spinal cord. Their determination was made by whole-body autoradiography. The highest concentration of DDT in the brain was localized in the gray matter. DDT was also found in the ovaries, particularly in the corpora lutea. It was shown that the DDT freely passed the placental barrier and became concentrated in the liver and adipose tissues of the fetus. After birth, the new born mice were allowed to suckle for a few days. They showed a somatic distribution of DDT similar to that of their mothers.

Good, Ware, and Miller (1965) reported a decrease in both size and numbers of test animals that were fed a diet containing 5 ppm of Kepone, another chlorinated hydrocarbon. Huber (1965) reported that a decrease in the litter sizes of animals treated with high levels of Kepone was due to a reduction in the amount of lutenizing hormone present. He theorized that the production of lutenizing hormones was inhibited by the high concentrations of the insecticide. Ware and Good (1967a) reported that when Mirex, a formicide and analog of Kepone, is incorporated in the diet of the mouse at 5 ppm, the litter size is definitely reduced. In the same experiment 7 ppm of DDT was included in the diet and caused a slight reduction in the size of the litters.

Ware and Good (1967b) observed no reproductive failures in mice which were treated with the carbamate insecticide Tranid, in which 5 ppm was mixed in the diet for a period of 120 days.

Research has also been conducted in an effort to observe the effects of endrin and dieldrin on reproduction in laboratory mice (Good and Ware, 1969). These insecticides were separately mixed in the diet at 5 ppm for 120 days. It was demonstrated that endrin produced significant parental mortality and a reduction in the size of the litters, whereas dieldrin caused no parental mortality, but did cause a reduction in the size of the litters.

Tarjan and Kemeny (1969) reported on multigeneration studies of mice that were fed 2.8 to 3.0 ppm of DDT in their daily diets. They concluded that the test and control groups showed no significant differences in the number of pregnancies, births, litters and surviving weanlings. The results of the Tarjan-Kemeny studies were in contrast to the work of Ware and Good (1967) with regard to DDT's effects on litter size. Deichman and Keplinger (1969) performed various experiments on five generations of laboratory mice using different concentrations of chlordane, DDT, aldrin, and dieldrin. They determined that these various mixtures produced some effects on reproduction, especially with regard to decreased viability.

CHAPTER III

MATERIALS AND METHODS

Materials

The research animals used were six-to eight-week old Swiss Webster mice. They were purchased from the Nasco-Steinhilber Company, Fort Atkinson, Wisconsin.

The mice were housed individually, or in small groups, in plastic animal cages with wire covers. The cages were 10 inches long, 6 inches wide, and 5 inches tall (25.4 x 15.2 x 12.7 cm.). Deodorized wood shavings were used as bedding. The mice were fed a diet of Purina laboratory chow and had constant access to water.

The chlordane administered is known as octachloro-4, 7-methanotetrahydroindane. It is available commercially under the trade name "Unico Termite Kill" and is produced by United Co-Operatives Incorporation of Alliance, Ohio. The active ingredients were chlordane (45 percent) and petroleum distillate (50 percent). The remainder (5 percent) consisted of inert ingredients. All testing was done with this commercial product.

Chlordane is a viscous liquid, insoluble in water, but soluble in most organic solvents. Because chlordane is insoluble in water, olive oil was selected as the carrier for all injections. One cubic centimeter (1cc) tuberculin syringes were used to administer the insecticide and carrier.

In order to obtain accurate animal weights, a triple beam balance with an accompanying animal box was employed.

Methods

Calculations of the Chlordane Concentrations

The administered chlordane concentrations were calculated in milligrams of chlordane per kilogram of body weight of the test animals (mg/kg). Because the chlordane was administered with one cc disposable syringes, it was necessary to determine the number of mg of chlordane contained in one cc. The commercial product used in this study contained four pounds of chlordane per gallon (1814.00 g/3785.40 cc), which is equivalent to 480 mg/cc. One cc of chlordane was mixed with nine cc of olive oil. Through dilution, more accurate measurements of the dose concentrations could be made.

The mg/kg values were calculated using proportionality as in the following example. If a 500 mg/kg concentration of chlordane was to be injected into a mouse that weighed 0.030 kg, then a proportion was constructed to find out the number of mg of chlordane equivalent to 500 mg/kg in a 0.030 kg mouse (500 mg/kg = Xmg/kg = Xmg/0.030kg). In this case the value of X equaled 15.0 mg. Because the stock solution contained 48 mg/cc, the exact dosage administered to obtain the 500 mg/kg ratio could be determined by employing the

formula 48 mg/cc = 15 mg/Y. The value of Y in this case equaled 0.30 cc. Thus, in a 1:9 solution of chlordane and olive oil, a 500 mg/kg concentration for a mouse that weighs 0.030 kg amounts to 0.30 cc.

Control animals were injected with 0.30 cc of the carrier substance olive oil. When the dosage for the experimental animals injected with the chlordane concentration was less than 0.30 cc, olive oil was added to the dosage until the total injected equaled 0.30 cc. Therefore, all animals were administered a constant volume of 0.30 cc.

Route of Administration

The chlordane was injected intraperitoneally with a one cc tuberculin syringe. This method of administration was patterned after the methods of Hart et al. (1962), Fouts and Rogers (1964), and Conney et al. (1965). The intraperitoneal route was used primarily because a more rigid control of the dosage could be maintained (Hart et al., 1962). Each syringe was disposed of immediately after use.

Preliminary Test to Obtain Sublethal Dose Levels

A preliminary test was conducted on nonpregnant Swiss Webster mice to obtain the sublethal dose levels of chlordane used in this study. There were eleven groups

of experimental animals plus one control group. Each group consisted of six animals. Dosages were initiated at 100 mg/kg and concluded at 500 mg/kg, spaced at 25 mg/kg intervals, in accordance with the minimum standards recommended by Hagan (1959).

Execution of the Study

After sublethal dose levels were determined, primary testing was initiated. Mature virgin female mice were placed in cages with male mice in late evening and checked for vaginal plugs the following morning. The presence of a vaginal plug is a reliable conception indicator. The pregnant mice were then randomly isolated into one of the nine different groups and injected with specific concentrations of chlordane at different intervals. Table I outlines the basic experimental design.

TABLE I

DOSE CONCENTRATIONS, TIME OF INJECTIONS, GROUP DIVISIONS AND NUMBERS FOR CONTROL AND EXPERIMENTAL GROUPS

	Time of Injection		
Dose	Early	Middle	Late
Control (Olive Oil)	Group 1	Group 2	Group 3
X mg/kg	Group 4	Group 5	Group 6
Y mg/kg	Group 7	Group 8	Group 9

The pregnant mice treated during the middle and late gestation periods were administered proportionate chlordane concentrations equivalent to those for 0.025 kg mice, determined by averaging the body weights of the 97 test animals on day one of pregnancy. This modified dose concentration was necessary because the excess weight gain (due to pregnancy) during the middle and late gestation periods was not comparable to the body weights of the mice treated during early gestation. The administration of truly proportionate chlordane concentrations would have resulted in abnormal maternal death rates (as determined by preliminary testing).

Swiss Webster mice have a relatively short gestation period of approximately 21 days; therefore, the earlier administration was either the first, second, or third day of gestation; the middle period was either the tenth, eleventh, or twelfth day of gestation; and the later period was either the sixteenth, seventeenth, or eighteenth day of gestation. These three gestation stages were selected for chlordane administration because they were considered critical in embryonic development. During the early period the fertilized ova is in the process of traveling down the fallopian tube prior to implantation in the uterus; rapid fetal development occurs during the middle period. The late period is critical because the organ system formation is near completion and birth is imminent.

CHAPTER IV

RESULTS AND DISCUSSION

Determination of Sublethal Dose Levels

Sublethal dose levels were determined by injecting nonpregnant mice with varying amounts of the insecticide. The results are contained in Table II. Within Table II the specific dose concentrations in mg/kg are listed along with the corresponding death and survival rates.

TABLE II

DOSE CONCENTRATIONS, SURVIVAL AND DEATH RATES OF NON-PREGNANT FEMALE MICE AFTER 21 DAYS

	Dose Concentrations (Mg/Kg)											
	100	200	225	250	275	300	325	350	375	400	500	Control
Died	0	0	0	1	2	2	2	2	2	4	6	0
Survived	6	6	6	5	4	4	4	4	4	2	0	6

A linear evaluation for the determination of the sublethal dose levels listed in Table II is presented in Figure 1. The number of deaths after a three week period are graphically depicted.

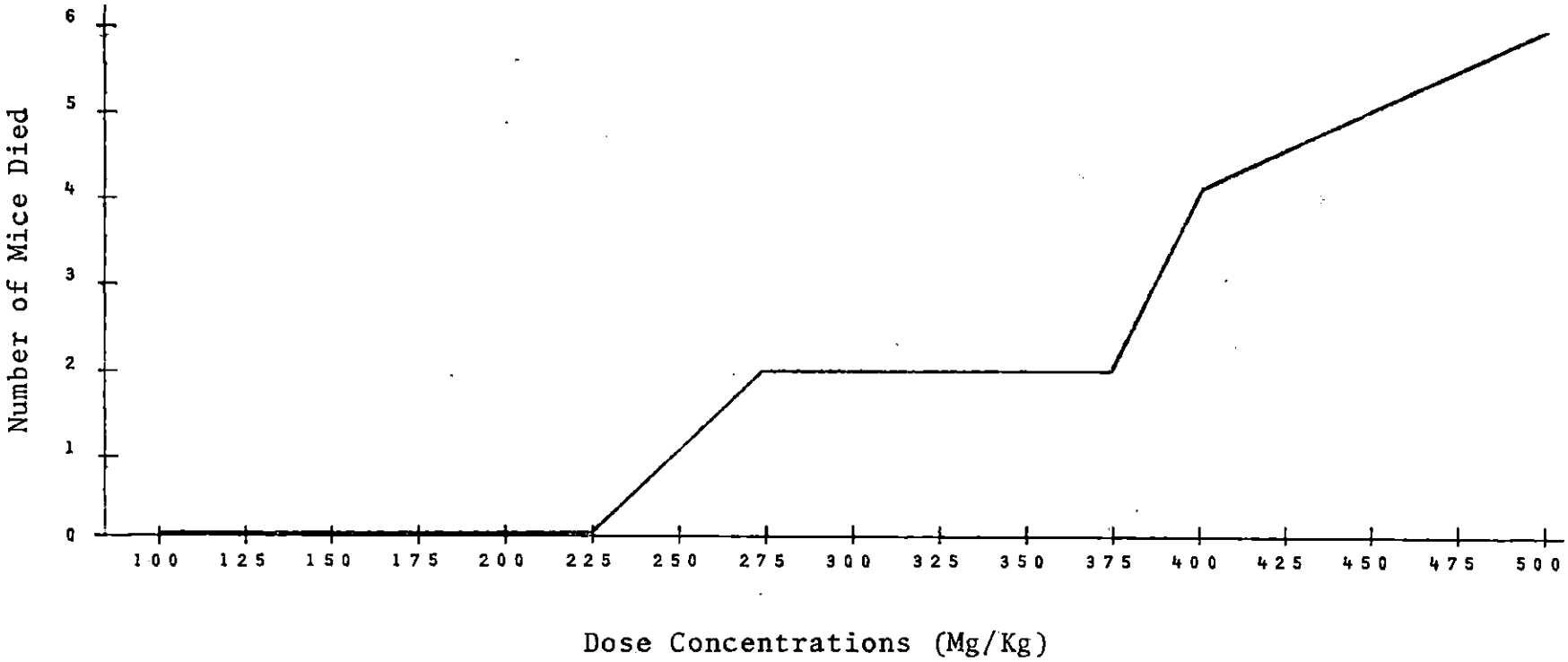

Figure 1. Number of mice that died per dose concentration of chlordane

Stohlman et al. (1950) found that the LD_{50} of pure chlordane injected intraperitoneally in rats was 200 mg/kg. Similarly Kenaga and Allison (1969) found the LD_{50} for pure chlordane through oral administrations to range from 283 to 590 mg/kg. Because of these findings, 25 mg/kg dose intervals were selected in the 200 to 400 mg/kg range to insure accurate evaluations of the sublethal dose levels.

The results indicate that survival is less than 50 percent near the 375 to 400 mg/kg range. The increase in death rates with increasing concentrations lends credence to the results.

The Effects of Sublethal Dose Levels

The sublethal dose levels of chlordane utilized in this portion of the research were 200 mg/kg and 300 mg/kg. The 200 mg/kg dose was selected because all preliminary test mice survived this dose; the 300 mg/kg dose was selected because it represented one point at which survival was slightly more than 50 percent, yet sufficiently distant from the critical range of 375 to 400 mg/kg. These two levels (200 and 300 mg/kg) replaced the X and Y mg/kg, respectively in Table I.

The research animals were injected with these insecticide concentrations during the previously designated periods of gestation. The results observed in nine experimental groups included the ability of the pregnant mice to survive, a comparison of litter sizes, survival

rate of the mouse pups until weaning and the frequency of maternal cannibalism. Tables III through XI contain these results.

TABLE III

Group 1

PARENT-SIBLING SURVIVAL RATES, LITTER SIZE, AND CANNIBALISM IN THE CONTROL GROUP INJECTED DURING THE EARLY PERIOD OF GESTATION

Individual Animals	Female Survival[a]	Litter Size	Number of Pups Died[b]	Number of Pups Cannibalized
1	+	12	0	12
2	+	9	0	9
3	+	10	0	0
4	+	6	0	0
5	+	9	5	0
6	+	9	0	9
7	+	8	0	0
8	-[c]	9	0	0
9	+	0	0	0
10	+	8	0	0
Percent of Total	90		2.5	37
Mean		8.8[d]		

[a]Female survival: + indicates female survived; - indicates female died.
[b]Number of pups died and Pup Survival (%) indicates the number of mouse pups that died due to causes other than cannibalism from the time of birth until weaning.
[c]Female died after parturition.
[d]Includes only those offspring of females which actually gave birth.

TABLE IV

GROUP 2

PARENT-SIBLING SURVIVAL RATES, LITTER SIZE, AND CANNIBALISM IN THE CONTROL GROUP INJECTED DURING THE MIDDLE PERIOD OF GESTATION

Individual Animals	Female Survival[a]	Litter Size	Number of Pups Died[b]	Number of Pups Cannibalized
1	+	11	0	11
2	+	6	1	0
3	+	11	3	0
4	+	13	3	0
5	+	12	2	0
6	+	12	1	0
7	+	8	0	0
8	+	11	2	0
Percent of Total	100		14.2	13
Mean		10.5[c]		

[a]Female survival: + indicates female survived; - indicates female died.

[b]Number of pups died and Pup Survival (%) indicates the number of mouse pups that died due to causes other than cannibalism from the time of birth until weaning.

[c]Includes only those offspring of females which actually gave birth.

TABLE V

Group 3

PARENT-SIBLING SURVIVAL RATES, LITTER SIZE, AND CANNIBALISM IN THE CONTROL GROUP INJECTED DURING THE LATE PERIOD OF GESTATION

Individual Animals	Female Survival[a]	Litter Size	Number of Pups Died[b]	Number of Pups Cannibalized
1	+	12	3	0
2	+	11	2	0
3	+	9	0	0
4	+	9	3	0
5	+	8	0	8
6	+	11	2	0
7	+	11	0	11
8	+	9	4	0
Percent of Total	100		17.5	23.7
Mean		10[c]		

[a]Female survival: + indicates female survived; - indicates female died.

[b]Number of pups died and Pup Survival (%) indicates the number of mouse pups that died due to causes other than cannibalism from the time of birth until weaning.

[c]Includes only those offspring of females which actually gave birth.

TABLE VI

Group 4

PARENT-SIBLING SURVIVAL RATES, LITTER SIZE, AND CANNIBALISM IN THE 200 Mg/Kg GROUP INJECTED DURING THE EARLY PERIOD OF GESTATION

Individual Animals	Female Survival[a]	Litter Size	Number of Pups Died[b]	Number of Pups Cannibalized
1	-	0	0	0
2	-	0	0	0
3	-	0	0	0
4	-	0	0	0
5	+	0	0	0
6	+	0	0	0
7	-	0	0	0
8	-	0	0	0
9	-	0	0	0
10	+	9	3	0
11	+	0	0	0
12	+	0	0	0
13	+	0	0	0
14	+	0	0	0
15	-	0	0	0
16	+	10	4	0
17	+	0	0	0
18	+	0	0	0
19	+	0	0	0
20	+	0	0	0
Percent of Total	60		36.8	0.0
Mean		9.5[c]		

[a]Female survival: + indicates female survived; - indicates female died.

[b]Number of pups died and Pup Survival (%) indicates the number of mouse pups that died due to causes other than cannibalism from the time of birth until weaning.

[c]Includes only those offspring of females which actually gave birth.

TABLE VII

Group 5

PARENT-SIBLING SURVIVAL RATES, LITTER SIZE, AND CANNIBALISM IN THE 200 Mg/Kg GROUP INJECTED DURING THE MIDDLE PERIOD OF GESTATION

Individual Animals	Female Survival[a]	Litter Size	Number of Pups Died[b]	Number of Pups Cannibalized
1	-	0	0	0
2	+	10	10	0
3	+	13	1	0
4	+	7	7	0
5	+	10	1	0
6	+	8	4	0
7	-	0	0	0
8	-	0	0	0
Percent of Total	62.5		47.9	
Mean		9.6[c]		0.0

[a] Female survival: + indicates female survived; - indicates female died.

[b] Number of pups died and Pup Survival (%) indicates the number of mouse pups that died due to causes other than cannibalism from the time of birth until weaning.

[c] Includes only those offspring of females which actually gave birth.

TABLE VIII

Group 6

PARENT-SIBLING SURVIVAL RATES, LITTER SIZE, AND CANNIBALISM IN THE 200 Mg/Kg GROUP INJECTED DURING THE LATE PERIOD OF GESTATION

Individual Animals	Female Survival[a]	Litter Size	Number of Pups Died[b]	Number of Pups Cannibalized
1	-c	7	0	7
2	+	10	10	0
3	+	12	12	0
4	+	10	1	0
5	-c	9	9	0
6	+	12	1	0
7	-c	7	7	0
8	-	0	0	0
Percent of Total	50		59.7	10.4
Mean		9.57[d]		

[a]Female survival: + indicates female survived; - indicates female died.

[b]Number of pups died and Pup Survival (%) indicates the number of mouse pups that died due to causes other than cannibalism from the time of birth until weaning.

[c]Female died after parturition.

[d]Includes only those offspring of females which actually gave birth.

TABLE IX

Group 7

PARENT-SIBLING SURVIVAL RATES, LITTER SIZE, AND CANNIBALISM IN THE 300 Mg/Kg GROUP INJECTED DURING THE EARLY PERIOD OF GESTATION

Individual Animals	Female Survival[a]	Litter Size	Number of Pups Died[b]	Number of Pups Cannibalized
1	-	0	0	0
2	+	0	0	0
3	-	0	0	0
4	-	0	0	0
5	-	0	0	0
6	-	0	0	0
7	-	0	0	0
8	-	0	0	0
9	-	0	0	0
10	-	0	0	0
11	-	0	0	0
12	-	0	0	0
13	-	0	0	0
14	+	0	0	0
15	-	0	0	0
16	-	0	0	0
17	-	0	0	0
18	-	0	0	0
19	-	0	0	0
Percent of Total	10.5		0.0	0.0
Mean		0		

[a] Female survival: + indicates female survived; - indicates female died.

[b] Number of pups died and Pup Survival (%) indicates the number of mouse pups that died due to causes other than cannibalism from the time of birth until weaning.

TABLE X

Group 8

PARENT-SIBLING SURVIVAL RATES, LITTER SIZE, AND CANNIBALISM IN THE 300 Mg/Kg GROUP INJECTED DURING THE MIDDLE PERIOD OF GESTATION

Individual Animals	Female Survival[a]	Litter Size	Number of Pups Died[b]	Number of Pups Cannibalized
1	-	0	0	0
2	-	0	0	0
3	-	0	0	0
4	+	0	0	0
5	+	0	0	0
6	+	0	0	0
7	+	0	0	0
8	+	0	0	0
Percent of Total	62.5		0.0	0.0
Mean		0		

[a]Female survival: + indicates female survived; - indicates female died.

[b]Number of pups died and Pup Survival (%) indicates the number of mouse pups that died due to causes other than cannibalism from the time of birth until weaning.

TABLE XI

Group 9

PARENT-SIBLING SURVIVAL RATES, LITTER SIZE, AND CANNIBALISM IN THE 300 Mg/Kg GROUP INJECTED DURING THE LATE PERIOD OF GESTATION

Individual Animals	Female Survival[a]	Litter Size	Number of Pups Died[b]	Number of Pups Cannibalized
1	+	12	2	0
2	+	9	9	0
3	+	9	9	0
4	+	11	1	0
5	+	9	0	9
6	+	12	1	0
7	-	0	0	0
Percent of Total	85.7		35.4	14.5
Mean		10.3[c]		

[a]Female survival: + indicates female survived; - indicates female died.

[b]Number of pups died and Pup Survival (%) indicates the number of mouse pups that died due to causes other than cannibalism from the time of birth until weaning.

[c]Includes only those offspring of females which actually gave birth.

The Survival Rate of the Pregnant Mice

The initial statistical analysis of the data in Tables III through XI was performed by means of a two way analysis of variance. This analysis was conducted to determine if any significant abnormalities could be attributed to the time of insecticide administration, the dose level (treatment) of the insecticide, or an interaction between the time of administration and the treatment. The results of the initial analysis pertaining to maternal survival are presented in Table XII.

TABLE XII

TWO WAY ANALYSIS OF VARIANCE TO DETERMINE THE ABILITY OF PREGNANT MICE TO SURVIVE SUBLETHAL CHLORDANE TREATMENTS DURING SPECIFIC TIMES OF GESTATION

Source of Variance	Sum of Squares	Degrees of Freedom	Mean Square	F_c	$F_{.95}$
Mean (μ)	37.11	1	37.11	228.09	3.96
Time (γ)	2.22	2	1.11	6.83	3.11
Treatment ($\times$)	4.17	2	2.08	12.81	3.11
Interaction	39.17	4	9.79	60.19	2.48
Error	14.39	88	.16		

Sum of Squares for Regression = SSR
Sum of Squares = SS
Time = SSR (μ,γ) - SSR (μ) = SS (Time adjusted for the mean)
Treatment = SSR (μ,γ,$\times$) - SSR (μ,γ) = SS (Treatment adjusted for the mean and time)

With the two way analysis of variance, if the F statistic for the sum of squares of the interaction proves significant then a general treatment effect between experimental and control groups can not be formulated. The F statistic was significant at the .05 level (Table XII, $F_c = 60.19$, $F_{.95} = 2.48$); hence, a comparison of the treatments at each time interval was performed because the treatment effect varied with the time of administration. A one way analysis of variance was performed on each time interval (early, middle, and late gestation) to determine exactly what results and corresponding time intervals were significantly effected by the treatment. Experimental and control groups were combined because of the high interaction. The results are presented in Tables XIII through XV.

TABLE XIII

ONE WAY ANALYSIS OF VARIANCE TO DETERMINE THE ABILITY OF PREGNANT MICE TO SURVIVE SUBLETHAL CHLORDANE TREATMENTS DURING THE EARLY PERIOD OF GESTATION

Source of Variance	Sum of Squares	Degrees of Freedom	Mean Square	F_c	$F_{.95}$
Between Means	4.71	2	2.36	14.48	3.20
Within Samples	7.49	46	.16		

TABLE XIV

ONE WAY ANALYSIS OF VARIANCE TO DETERMINE THE ABILITY OF PREGNANT MICE TO SURVIVE SUBLETHAL CHLORDANE TREATMENTS DURING THE MIDDLE PERIOD OF GESTATION

Source of Variation	Sum of Squares	Degrees of Freedom	Mean Square	F_c	$F_{.95}$
Between Means	.75	2	.37	2.10	3.47
Within Samples	3.75	21	.18		

TABLE XV

ONE WAY ANALYSIS OF VARIANCE TO DETERMINE THE ABILITY OF PREGNANT MICE TO SURVIVE SUBLETHAL CHLORDANE TREATMENTS DURING THE LATE PERIOD OF GESTATION

Source of Variation	Sum of Squares	Degrees of Freedom	Mean Square	F_c	$F_{.95}$
Between Means	1.05	2	.53	3.70	3.49
Within Samples	2.85	20	.14		

The data presented in Tables XIII through XV identify the gestation periods in which pregnant mice are more vulnerable to chlordane treatments. The analyses indicate that the survival rate of the experimental pregnant mice when compared to the survival rate of the control group was significantly affected by chlordane treatments administered during the early and late periods of gestation. No significant decrease in maternal survival occurred when treatments were administered during the middle period of gestation.

The high maternal death rate (53 percent) that occurred during the early period of gestation was unexpected. This rate was far greater than that for the middle and late periods of gestation (25 and 22 percent, respectively). A possible reason for the high death rate during the early period was that the mice weighed less than their counterparts in later stages of pregnancy. Therefore, the chlordane was distributed throughout the mice themselves, rather than throughout the mice and their embryos. The mice in late stages of pregnancy, in effect, received less chlordane per unit of maternal body weight than did the mice in the earliest stages of pregnancy.

The one way analysis of variance parameters presented in Table XV show that the survival rate for pregnant mice treated during the late gestation period was significant at the .05 level, whereas the survival rate was not significant in the middle stage of pregnancy

(Table XIV, $F_c = 2.10$, $F_{.95} = 3.47$). The significance of the late gestation period survival rate, however, was marginal and it is felt that a larger sample size may have changed the results considerably because the general trends are evident.

When results were analyzed in this perspective, it became evident that chlordane treatments administered to pregnant mice during the early gestation periods affected maternal survival far more than chlordane treatments administered during either the middle or late gestation periods.

The Survival Rate of the Mouse Pups from Parturition until Weaning

A statistical analysis was performed to determine the survival rate of the mouse pups whose maternal parents were administered sublethal chlordane treatments during the previously specified periods of gestation. For the basic data refer to Tables III through XI. The results of the analysis are presented in Table XVI.

TABLE XVI

TWO WAY ANALYSIS OF VARIANCE TO DETERMINE THE SURVIVAL RATE OF THE MOUSE PUPS WHOSE MATERNAL PARENTS WERE ADMINISTERED SUBLETHAL CHLORDANE TREATMENTS DURING SPECIFIC TIMES OF GESTATION

Source of Variation	Sum of Squares	Degree of Freedom	Mean Square	F_c	$F_{.95}$
Mean (μ)	3.75	1	3.75	57.87	4.10
Time (γ)	.41	2	.21	3.20	3.25
Treatment ($\varkappa$)	1.36	2	.68	10.52	3.25
Interaction	1.58	2	.78	12.16	3.25
Error	2.46	38	.06		

Sum of Squares for Regression = SSR
Sum of Squares = SS
Time = SSR (μ,γ) - SSR (μ) = SSR (Time adjusted for the mean)
Treatment = SSR ($\mu,\gamma,\varkappa$) - SSR (μ,γ) = SS (Treatment adjusted for the mean and time)

Because the sum of squares for interaction was significant, a one way analysis of variance was performed on each time interval (gestation period) to determine the sibling survival rates significantly effected by the treatment.

TABLE XVII

ONE WAY ANALYSIS OF VARIANCE TO DETERMINE THE SURVIVAL RATE OF THE MOUSE PUPS WHOSE MATERNAL PARENTS WERE ADMINISTERED SUBLETHAL CHLORDANE TREATMENTS DURING SPECIFIC TIMES OF GESTATION

Source of Variation	Sum of Squares	Degrees of Freedom	Mean Square	F_c	$F_{.95}$
Between Means	.15	1	.15	7.65	5.12
Within Samples	.18	9	.02		

TABLE XVIII

ONE WAY ANALYSIS OF VARIANCE TO DETERMINE THE SURVIVAL RATE OF THE MOUSE PUPS WHOSE MATERNAL PARENTS WERE ADMINISTERED SUBLETHAL CHLORDANE TREATMENTS DURING THE MIDDLE PERIOD OF GESTATION

Source of Variance	Sum of Squares	Degrees of Freedom	Mean Square	F_c	$F_{.95}$
Between Means	.47	1	.47	5.92	4.84
Within Samples	.90	11	.08		

TABLE XIX

ONE WAY ANALYSIS OF VARIANCE TO DETERMINE THE SURVIVAL RATE OF THE MOUSE PUPS WHOSE MATERNAL PARENTS WERE ADMINISTERED SUBLETHAL CHLORDANE TREATMENTS DURING THE LATE PERIOD OF GESTATION

Source of Variation	Sum of Squares	Degrees of Freedom	Mean Square	F_c	$F_{.95}$
Between Means	.67	2	.34	2.12	3.55
Within Samples	2.84	18	.16		

These results indicate that the survival rate of the mouse pups was adversely effected when the chlordane treatments were administered during the early and middle periods of gestation (early, Table XVII, $F_c = 7.65$, $F_{.95} = 5.12$; middle, Table XVIII, $F_c = 5.92$, $F_{.95} = 4.84$). No decrease in sibling survival was attributed to chlordane during the late period of gestation (late, Table XIX, $F_c = 2.12$, $F_{.95} = 3.55$).

Any decrease in sibling survival may be attributed to the fact that chlorinated insecticides are capable of passing the placental barrier and become concentrated in the fetus [Backstrom et al. (1964); O'Leary et al. (1970); and Curley, Copeland, and Kimbrough (1969)]. Maternal mice administered chlordane during the early and middle periods of gestation had a longer amount of time for the pesticide to pass the placental barrier and become established in the fetuses than did the animals treated during the late gestation period. Thus, the offspring of the mice injected during the early and middle gestation periods should have higher chlordane concentrations than the offspring of the mice injected during the late gestation period and this could be a factor in their survival. Chlorinated insecticides also become concentrated in the mammary glands (Backstrom et al., 1964 and Ehrlich and Ehrlich, 1970). Thus, when the newborn are allowed to suckle they receive additional amounts of the insecticide.

With these two factors, it seems plausible that insecticide concentrations in the offspring could attain toxic levels. Thus, a reduction in the survival rate of the offspring could result.

A Comparison of the Litter Sizes

A statistical analysis was performed to determine significant differences in the numerical size of the litter in relation to the chlordane treatments administered to the maternal parents during the specified gestation periods. The results of the analysis are presented in Table XX; the basic data can be found in Tables III through XI.

TABLE XX

TWO WAY ANALYSIS OF VARIANCE TO DETERMINE SIGNIFICANT DIFFERENCES IN LITTER SIZES DUE TO THE CHLORDANE TREATMENT ADMINISTERED TO THE MATERNAL PARENT DURING SPECIFIC PERIODS OF GESTATION

Source of Variation	Sum of Squares	Degrees of Freedom	Mean Square	F_c	$F_{.95}$
Mean (μ)	4302.22	1	4302.22	1241.43	4.10
Time (γ)	9.13	2	4.56	1.32	3.25
Treatment ($\times$)	2.56	2	1.28	.37	3.25
Interaction	2.44	2	1.22	.35	3.25
Error	131.63	38	3.46		

Sum of Squares for Regression = SSR
Sum of Squares = SS
Time = SSR (μ,γ) - SSR (μ) = SSR (Time adjusted for the mean)
Treatment = SSR ($\mu,\gamma,\times$) - SSR (μ,γ) = SS (Treatment adjusted for the mean and time)

There was no significance at the .05 level in the analysis presented in Table XX. The non-significance indicated that litter sizes were not affected by the administration of sublethal concentrations of chlordane to the maternal parents during either early, middle, or late gestation.

The Frequency of Maternal Cannibalism

A statistical analysis was performed to determine if the frequency of maternal cannibalism was significantly effected by the chlordane administrations to the maternal parent during specific periods of gestation. The results of the analysis are presented in Table XXI; the basic data can be found in Tables III through XI.

TABLE XXI

TWO WAY ANALYSIS OF VARIANCE TO DETERMINE THE FREQUENCY OF MATERNAL CANNIBALISM DUE TO THE CHLORDANE TREATMENT ADMINISTERED TO THE MATERNAL PARENT DURING SPECIFIC PERIODS OF GESTATION

Source of Variation	Sum of Squares	Degrees of Freedom	Mean Square	F_c	$F_{.95}$
Mean (μ)	1.42	1	1.42	8.91	4.10
Time (γ)	.23	2	.12	.73	3.25
Treatment ($\times$)	.21	2	.11	.67	3.25
Interaction	.06	2	.03	.19	3.25
Error	6.06	38	.16		

Sum of Squares for Regression = SSR
Sum of Squares = SS
Time = SSR (μ,γ) - SSR (μ) = SSR (Time adjusted for the mean)
Treatment = SSR ($\mu,\gamma,\times$) - SSR (μ,γ) = SS (Treatment adjusted for the mean and time)

There was no significance at the .05 level in the analysis presented in Table XXI. This indicated that the frequency of maternal cannibalism was not affected by the administration of chlordane treatments to the maternal parents during either early, middle, or late gestation.

CHAPTER V

SUMMARY AND CONCLUSIONS

This study of the effects of chlordane on pregnant mice yielded several conclusions, numerically listed below.

1. Chlordane treatments administered to pregnant mice during the early gestation periods affected maternal survival far more than chlordane treatments administered during either the middle or late gestation periods.
2. The sibling survival rate was adversely effected when the chlordane treatments were administered during the early and middle periods of gestation.
3. The litter sizes were not affected by the administration of sublethal concentrations of chlordane to the maternal parent during either early, middle, or late gestation.
4. The frequency of maternal cannibalism was not affected by the administration of chlordane during early, middle, or late gestation.
5. No teratogenic defects were observed among the offspring whose maternal parents received chlordane treatments.

There is a definite need for additional research pertaining to the effects of insecticides on pregnant mammals. Future investigations are especially needed to determine if numerous pesticides administered simultaneously act synergistically to adversely effect pregnant mammals and their offspring. Another aspect warranting investigation is concerned with the mode of chlordane transmission and distribution in pregnant mice and their fetuses. A corollary investigation could be concerned with organ and membrane concentrations. Another possible investigation could be concerned with the effects of the petroleum distillate alone. A study on the effects of chlordane or other insecticides could be performed on maternal mice in early gestation. This period seems very critical.

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